EXHIBIT L

IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA, ex rel,   '
W. A. DREW EDMONDSON,   '
in his capacity as ATTORNEY GENERAL   '
OF THE STATE OF OKLAHOMA,   '
and OKLAHOMA SECRETARY   '
OF THE ENVIRONMENT   '
C. MILES TOLBERT, in his capacity as   '
the TRUSTEE FOR NATURAL RESOURCES   '
FOR THE STATE OF OKLAHOMA,   '
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     Plaintiffs,   ' Case No. 4:05-cv-00329-GKF-SAJ
                                                    '
vs.   '
                                                    '
TYSON FOODS, Inc.,   '
TYSON POULTRY, INC.,   '
TYSON CHICKEN, INC.,   '
COBB-VANTRESS, INC.,   '
AVIAGEN, INC.,   '
CAL-MAINE FOODS, INC.,   '
CAL-MAINE FARMS, INC., CARGILL, INC.,   '
CARGILL TURKEY PRODUCTION, LLC,   '
GEORGE'S, INC., GEORGE'S FARMS, INC.,   '
PETERSON FARMS, INC.,   '
SIMMONS FOODS, Inc.   '
WILLOWBROOK FOODS, INC.   '

     Defendants.

EXPERT REPORT OF VALERIE J. HARWOOD, Ph.D.

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IV. WATER QUALITY IN THE ILLINOIS RIVER WATERSHED

28. The IRW in Oklahoma hosts an intricate network of tributaries to the Illinois River, including Sager Creek, Flint Creek, Peacheater Creek, Tyner Creek, Tahlequah Creek and the Baron Fork of the Illinois River. The State of Oklahoma defines impaired waters as those in which "...the water quality standard is not attained. The water body is impaired or threatened for one or more designated uses by a pollutant(s)..." (State of Oklahoma, 2006b). Indicator bacteria levels in each of these tributaries routinely exceed Oklahoma water quality standards, therefore these water bodies have been placed on the State's 303(d) list of impaired waters. This Oklahoma Scenic River is considered to be too polluted by fecal bacteria to support its designated use of primary body contact recreation. Dr. Teaf's Expert Report for this case describes the extent of impairment in the IRW; in summary over 75% of the Illinois River and its major tributaries are listed as impaired by high bacterial levels (Teaf, 2008).

29. The data collected by the State of Oklahoma for water quality assessment includes (but is not limited to) fecal coliform concentrations. Fecal coliforms are used by the State of Oklahoma to evaluate recreational water quality, but are not recommended by the U.S. EPA due to their lack of correlation with human illness in some locations (U.S. Environmental Protection Agency, 1986). However, *E. coli* is recommended for recreational water quality monitoring by the U.S. EPA. A comparison of *E. coli* and fecal coliform concentrations in water samples collected throughout the IRW shows that almost all of the fecal coliforms in these samples are *E. coli* (Figure 1). This relationship confirms the public health significance of elevated fecal coliform concentrations in IRW waters, i.e. they are nearly synonymous with *E. coli* concentrations, which are correlated with the risk of gastroenteritis for recreational water users.

30. Enterococci are responsible for many of the water quality exceedances throughout the IRW (Teaf, 2008). This group of fecal indicator bacteria is recognized as measure of recreational water quality by the U.S. EPA and the State of Oklahoma, and its levels are correlated with the risk of gastroenteritis in recreational water users in fresh and salt water (Teaf, 2008).

31. The State of Oklahoma recognizes the potential impact of poultry operations and other agriculture on water quality. Under the Oklahoma Registered Poultry Feeding Operation Act, it is required that "...there shall be no discharge to waters of the state." (Title 2; Registered Poultry Feeding Operation Act) Management of poultry litter/manure in the IRW is by land application, which is considered a passive waste management approach that can impact

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surface and ground water quality as microorganisms move with surface and subsurface water flow (U.S. Environmental Protection Agency, 2005a). Broiler production generates large amounts of contaminated litter, i.e. up to 0.5 pounds of soiled litter per pound of meat produced, or 340 tons annually from a farm with only four houses (Dozier, Lacy & Vest, 2001). Used poultry litter is known to contain high levels of indicator bacteria. Contaminated poultry litter samples were collected by CDM from poultry houses in the IRW in 2006 (Camp Dresser & McKee (CDM), 2008). Ten samples, each from a different facility, were tested for indicator bacteria levels and for a poultry-specific biomarker (the biomarker is discussed in the Microbial Source Tracking Section below). The indicator bacteria concentrations in these samples were generally extremely high, with a geometric mean of ~1200 *E. coli* per gram of litter, and ~51,000 enterococci/g litter. The maximum levels for both indicator bacteria from any one location were over 100,000/g litter (Camp Dresser & McKee (CDM), 2008). *Salmonella* was detected in four of 24 contaminated poultry litter samples (16.7%), but *Campylobacter* was not detected by the culture-based methods used. More sensitive PCR methods that could detect viable but nonculturable pathogens would have been more suited to the detection of pathogens such as *Salmonella* and *Campylobacter* in poultry litter and environmental samples. Given the near-ubiquitous association of these pathogens with poultry feces, my opinion is that these pathogens were present, but that too few were present in a culturable state to be detected by the methods used, which were developed for the food industry and not for environmental samples where pathogens are physiologically stressed.

32. The anticipated pathway of surface water contamination from land-applied poultry litter would begin with runoff from the edges of fields on which litter had been spread. "Edge-of-field" samples collected by CDM in the IRW typically had very high levels of indicator bacteria (Camp Dresser & McKee (CDM), 2008). Some samples had *E. coli* levels of over 1 million/100 ml, which approaches the concentration found in raw sewage (Harwood et al., 2005). Soil samples collected from fields on which poultry litter had been land-applied as levels of up to 2,000 *E. coli* per gram of soil and 17,000 enterococci/g. As expected, IRW surface water samples had variable indicator bacteria levels; however, chronic exceedances of the primary body contact standard for bacteria levels were recorded throughout the IRW (detailed in Teaf, 2008). The data indicate that human exposure to fecal bacteria is occurring since the exceedances also occurred frequently at established "put-in" spots along the IRW, where people enter the water to swim, float, canoe or kayak.

33. Below the surface layer of soil in the IRW is a karst substratum that is riddled with cracks and fissures (Fisher, 2008). The effect of this karst terrain is that surface water and groundwater have a much greater physical connection than they do in other geological formations, and contaminants from the surface, including bacteria, can readily penetrate the shallow aquifer, and from there can find their way to deeper aquifers such as those used for drinking water (Davis, Hamilton & Van Brahana, 2005). Evidence for the widespread influence of surface contamination on groundwater quality is that indicator bacteria were isolated from springs, shallow wells and deep wells in the IRW (detailed in Teaf, 2008). Almost 1700 wells are registered for drinking water use in the Oklahoma portion of the IRW (Fisher, 2008). The owners of these wells generally do not disinfect or otherwise treat the water from the wells, therefore people can be exposed to pathogens that infiltrate the groundwater via runoff from fields on which poultry waste has been land-applied.

34. From 2000-2007 over one billion birds (chickens and turkeys) were produced by the defendants in the IRW (Fisher, 2008), or an average of over 141 million bird/year. In 2005-2006 there were over 1,900 active poultry houses in the IRW, generating an estimated 354,000 tons of waste (Fisher, 2008). Using the geometric mean values obtained from sampling poultry litter in the IRW shown above (and the knowledge that there are 907,184 g in a ton), the annual estimate of poultry litter-associated *E. coli* is 3.9 X 10^{14} cells (390 trillion), while for enterococci it is 1.6 X 10^{16} (16,000,000,000,000,000) cells. This material is spread on fields, generally within three to five miles of the area where it was produced, where it can leach into groundwater and run off into surface water (Fisher, 2008).

V. SPECIFIC EVIDENCE OF POULTRY FECAL CONTAMINATION IN THE IRW

35. Chemical/bacterial signal determined by principle components analysis.

Analysis of an array of chemical and bacterial parameters using the multivariate statistical method of principle components analysis has revealed a distinctive "signature" that is characteristic of soils and waters contaminated by poultry waste (Olsen, 2008). The measured parameters included metals, nutrients, physical measurements and indicator bacteria. A definitive poultry waste signature was derived from phosphorus, bacteria, organic carbon, potassium, copper, zinc, and nitrogen-containing compounds. The poultry waste signature was found in all sample types throughout the IRW, including edge-of-field, soils impacted by land application, rivers, streams, and their sediments, groundwater, and Lake Tenkiller. Olsen concluded that a significant source of bacterial contamination in the IRW was poultry waste, and that the signature was present at every leg of the transport pathway from litter to soil to edge-of-

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field samples to surface water and ground water (Olsen, 2008). This finding is consistent with my own opinion that land application of poultry litter is a dominant source of bacterial contamination to IRW surface waters and groundwater.

36. Bacterial loading in the IRW.

An analysis of fecal coliform loading from various potential sources in the six counties that contribute to the IRW was conducted for this investigation (Teaf, 2008). Pets, deer and wildlife, and human sources (i.e. septic systems, wastewater treatment plant discharges) together accounted for an estimated 1.4% of total loading of fecal coliforms to the IRW, while livestock accounted for 98.6%. Poultry and cattle contributed an approximately equal, major load (estimated at 41% and 44% of all livestock contributions, respectively). Contaminated poultry litter and soil receiving land-applied poultry litter contains an even higher load of enterococci than fecal coliforms; thus poultry are doubtless a dominant source of fecal indicator bacteria to the IRW.

37. Microbial source tracking.

Fecal coliforms, *E. coli* and enterococci are broad, nonspecific indicators of fecal pollution because they are shed in the feces of almost all warm-blooded animals. Certain animals, such as poultry, frequently harbor human pathogens in addition to indicator bacteria in their gastrointestinal tract (U.S. Environmental Protection Agency, 2005a; U.S. Environmental Protection Agency, 2007). Because the detection of high-risk fecal contamination and its discrimination from other sources of indicator bacteria is needed to inform management decisions and risk assessment, source-specific testing methodologies have been developed and validated (Stoeckel & Harwood, 2007; U.S. Environmental Protection Agency, 2005b). A number of approaches, collectively termed microbial source tracking (MST) methods, have been the subject of investigation and research by many investigators across the country, including U.S. EPA scientists (Santo-Domingo & Sadowsky, 2007; Stoeckel & Harwood, 2007; U.S. Environmental Protection Agency, 2005b; U.S. Environmental Protection Agency, 2007).

38. MST methods can be roughly grouped into library-dependent and library-independent approaches. **Library-dependent** methods typically begin by culturing, or growing, indicator bacteria such as *E. coli* or enterococci from the feces or sewage of various host species (e.g. chickens, cattle, humans) that may impact water quality in the study area. The isolates are typed, or "fingerprinted" by highly discriminatory laboratory methods, and their fingerprints make up the known source library. Fingerprinting can be carried out by a variety of

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46. *Validation of target sequence.* A PCR primer set was developed for each of the four potential targets (three bacterial and one *E. coli*) (Table 1). To increase the sensitivity of detection, a nested PCR approach was employed in which DNA was first amplified using universal bacterial primers (or all-*E. coli* primers) followed by amplification of an internal fragment with the target primers. Assay sensitivity was tested against composite poultry litter samples and against soil samples on which poultry litter had been land-applied. Specificity of the assays was tested against fecal samples from beef and dairy cattle, swine, ducks, geese, and human sewage. The collection and handling of these fecal samples is detailed in Dr. Olsen's report (Olsen, 2008), but a brief description of the makeup of these samples is below.

47. Nontarget fecal samples (from animals other than poultry and human sewage) for specificity testing were collected as composites from groups of individuals (Table 2). Beef cattle fecal samples were collected from ten grazing fields, of which five were within the watershed and five were outside the watershed. Two independent duplicate samples were collected for each field, and each duplicate consisted of feces from ten scats (feces from ten scats = 1 composite). A total of 200 beef cattle scats were tested. Duck (5 composites) and goose (5 composites) fecal samples were collected in the same fashion, consisting of composites from ten individual scats, and independent duplicates were collected for each area (Table 2). For ducks, three landing areas inside the watershed and two outside the watershed were sampled, while for geese, two landing areas inside and three landing areas outside the watershed were sampled. Composite samples of fecal slurries were collected from swine facilities, one inside the watershed and one outside (2 duplicate samples/facility) and dairy cattle farms (one inside the watershed and two outside (2 duplicate samples per facility) human residential septic cleanout tanks (3 samples) and influent of three separate municipal wastewater treatment plants (3 samples). A total of 20 g of each fecal sample from each site was collected and was placed in a 20 ml, sterile, polystyrene tube containing 10 ml of 20% glycerol and shipped on dry ice to the laboratory.

48. The PCR assay with greatest sensitivity (consistently able to detect the target in contaminated samples and specificity (lack of detection in non-target samples) was produced by primer set LA35, which targets a 16S rRNA gene fragment of 571 base pairs that is 98% identical to the DNA of *Brevibacterium avium*. The sequence was detected in all litter samples, and in eight of ten contaminated soil samples. Among the non-target fecal samples, it was only detected in one composite goose and one composite duck sample, each of which was collected outside the IRW (Table 2). Furthermore, the PLB was detected in only one of two duplicate

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samples from the cross-reactive duck and goose fecal composite, showing that it was present at low concentration in these samples.

49. PCR Validation Summary

- The nested PCR assay detected the PLB in all contaminated poultry litter samples, and in 80% of soils sampled from fields that received land-applied poultry. These tests indicated the method's sensitivity.

- The nested PCR assay did not detect the PLB in any of the nontarget fecal samples from the IRW, and found the target in low concentration (1 of 2 duplicates) from one duck and one goose sample collected outside the IRW. These tests indicated the method's specificity.

50. *Quantitative PCR.* A quantitative PCR (QPCR) assay was developed for the PLB using the LA35 primer set and Sybr green chemistry. This particular QPCR chemistry has the major advantage of allowing the production of a melting curve, which is determined by the temperature at which the double-stranded DNA of the PCR product melts and becomes single-stranded. Because the melting curve is particular to a given DNA sequence, this analysis allows a check of the purity and the identity of the QPCR product, which is particularly useful when analyzing environmental samples.

51. A QPCR assay should have a linear response to increasing concentrations of its target; in other words, the more copies of the gene are present, the more rapidly the signal rises. The precise quantitative nature of the PLB is demonstrated in Figure 3, which is a graph of crossing time (C_t) vs. gene copies of PLB. Crossing time is the time (generally in minutes) required until the fluorescent signal crosses a threshold above background levels, and is inversely proportional to gene copy number (the time required for the signal to rise above background levels is less as the concentration of target increases). The PLB gene fragment cloned into a plasmid was used as the template for the standard curve (Figure 3). The slope of the graph is negative (decreasing from left to right) because the C_t (time required to detect fluorescence) decreases with increasing concentrations of target DNA (in this case the PLB)

52. Although the same primers and annealing conditions (60° C) were used for both conventional nested PCR and QPCR, a number of fecal samples were re-tested by QPCR for specificity, including the goose and duck duplicate that were each found to be positive by the ultra-sensitive nested PCR. Table 3 contains results for previously tested samples (conventional

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nested PCR) that were re-tested for specificity. Each of these samples was below detection limit, or negative by QPCR, including the duck and goose sample that were positive by conventional nested PCR. Seven newly-collected beef cattle samples (Camp Dresser & McKee (CDM), 2008) were assayed and three uncontaminated (clean) poultry litter samples were tested (Table 4). Each of these control (clean poultry litter) and non-target samples gave results of "below detection limit" (BDL). In other words, a QPCR signal was not present in non-target animal fecal samples and clean litter. These results confirm the specificity of the PLB QPCR assay.

53. The concentration of fecal indicator bacteria in used poultry litter was compared to the concentration of the PLB to establish the relationship between the indicator organisms of fecal contamination and the poultry-specific marker. Enterococci concentrations were strongly and very significantly correlated with the PLB (r^2 = 0.7471; P = 0.013) (Figure 4), and *E. coli* concentrations also had a positive relationship with PLB concentration (r^2 = 0.3946; P = 0.052). The correlation of the poultry-specific PLB with the general fecal bacteria indicators provides confidence that co-contamination of waters with both types of indicators is common, and that they indicate a substantial health threat to recreational water users due to the known association of pathogens such as *Campylobacter* and *Salmonella* with poultry feces.

54. The QPCR assay for the PLB was field-tested on litter, soil and water samples, including edge-of-field, surface water and ground water samples. A total of ten soiled litter samples, 187 water samples and 40 soil samples were tested. Three of the water samples (BS-REF; Table 4) were collected outside of the IRW where used poultry litter is not land-applied; therefore they represent reference water samples which should not contain the PLB. In fact, the PLB in each of these samples was not detected in the negative control (reference) samples (Table 4). All contaminated litter samples contained very high concentrations of the PLB, ranging from 2.2×10^7 - 2.5×10^9 (tens of millions to billions) gene copies/g (Table 5). The PLB was at high enough concentration to be quantified by QPCR in 34 water samples, including 16 edge-of-field samples (Table 5), one groundwater sample (56287-7-13-06) and one spring sample (LAL15SP2-7-11-06). Six soil samples had quantifiable levels of the PLB, with the greatest at 3.8×10^6 gene copies/ml. Figures 5 and 6 show the results of QPCR testing for the PLB in water and soil samples, respectively. The level of quantified PLB for each site (location) is designated by a colored circle. Note that several sites were sampled more than once, so that the number of data points is fewer than the total number of samples in which the PLB was quantified.